PTHrP/PTH1R Signaling in Bone Marrow Adipocytes Regulates Maternal Skeletal Adaptation During Lactation

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INTRODUCTION: A reciprocal relationship between bone and bone marrow adipose tissue (BMAT) has been well-documented in numerous studies, highlighting their antagonistic interactions. Interestingly, we and others have discovered an unexpected, concordant loss of both bone tissue and BMAT during lactation, when the skeleton is subjected to significant stress due to maternal calcium homeostasis and energy metabolism [1,2]. Estrogen deficiency and elevated serum parathyroid hormone-related peptide (PTHrP) are consistently observed during lactation, suggesting their involvement in these metabolic processes [3,4]. Therefore, the objective of this study is to elucidate the effects of these two key endocrine signals on bone marrow adipocytes (BMAds) and to explore the interaction between bone and BMAT. Based on findings that constitutive PTHrP leads to reductions in both bone and BMAT, we further hypothesize that PTHrP/PTH1 receptor (PPR) signaling in BMAds plays a crucial role in regulating bone-BMAT adaptations in the maternal skeleton.

METHODS: All animal experiments were IACUC approved. First, 2-month-old female B6 mice were divided into three groups (n=4-5/group): Virgin, 12-day Lactation, and 14-day Recovery. Femurs were harvested, scanned using micro-computed tomography (μCT) for trabecular and cortical bone morphometry, and analyzed for perilipin^{+} mature BMAd fraction (Ad.Ar/Ma.Ar) and area (Ad.Ar). In the second study, 6-month-old female CD1 mice were continuously infused with either vehicle (VEH, 2% L-cysteine) or PTHrP1-34 (PTHrP, 34 μg/kg/day) via osmotic pumps (Alzet) for 2 weeks (n=5/group). Distal femurs were scanned with *in vivo* μCT before and after infusion to assess trabecular bone morphometry. Additionally, 10-month-old female CD1 mice underwent ovariectomy (OVX) and were euthanized 6 weeks later, alongside age-matched intact (INT) controls. Right tibiae from the PTHrP and OVX experiments were analyzed for BMAd fraction, area, and perimeter (Ad.Pm). Finally, 2-month-old female Adipoq-CreERT2; PPR^{floxed} (cKO) and wildtype (WT) littermate mice were divided into virgin and lactation groups. All mice received tamoxifen at 7.5 weeks of age. Lactating mice were mated 3 weeks post-tamoxifen and euthanized at 12d lactation, with age-matched WT mice euthanized concurrently. Left proximal tibiae were μCT scanned *ex vivo* for trabecular bone morphometry, and the midshaft was analyzed for cortical thickness and porosity. Right proximal tibiae were analyzed for BMAd fraction, area, and perimeter. Statistical significance was determined using one-way ANOVA or Student's t-test with Bonferroni correction.

RESULTS: (1) No statistically significant difference was found in BV/TV. Significantly lower Tb.Th and Ct.Th were observed in lactating mice compared to virgins (Fig. 1A-C, -30% and -18%, respectively), while post-weaning mice had 25% and 20% higher Tb.Th and Ct.Th, respectively. Compared to virgin mice, lactating mice showed a non-significant difference in adipocyte fraction and a 34% smaller size (Fig. 1D-E). Following recovery, both adipocyte fraction and size were significantly larger by 520% and 183%, respectively, compared to lactating mice. **(2)** The continuous infusion of either the vehicle or PTHrP led to significant reductions in BV/TV by 33% and 37%, respectively (Fig. 2A). While the PTHrP infusion resulted in a 12% decrease in Tb.Th, this reduction was not observed in the VEH group. Additionally, the PTHrP mice showed smaller adipocyte fraction, size and perimeter (Fig. 2C-E, -62%, -48%, and -24%, respectively). In contrast, the OVX mice showed reduced BV/TV by 25% without affecting Tb.Th (Fig. 2F-G) compared to the intact group. The OVX mice exhibited a 10-fold greater adipocyte fraction, while size and perimeter showed non-significance (Fig. 2H-J). **(3)** While both the WT and the Adipoq-CreERT2;PPR cKO mice displayed significantly lower BV/TV at 12d lactation (Fig. 3A, -32% and -28%, respectively), only the WT mice showed significantly lower Tb.Th (-29%) and Ct.Th (-12%), along with greater Ct. porosity (23%) at 12d lactation (Fig. 3B-D). In contrast, the cKO mice did not exhibit differences in these structural parameters. In addition, both groups showed significantly smaller adipocyte fraction during lactation (-92% and -74%, respectively; Fig. 3F), with no significant change in adipocyte size or perimeter in either group (Fig. 3G-H).

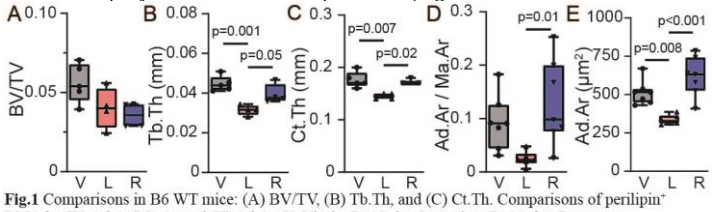

Fig.1 Comparisons in B6 WT mice: (A) BV/TV, (B) Tb.Th, and (C) Ct.Th. Comparisons of perilipin^{+} BMAds: (D) Ad.Ar/Ma.Ar and (E) Ad.Ar. V: Virgin, L: 12-day Lactation, R: 14-day Recovery

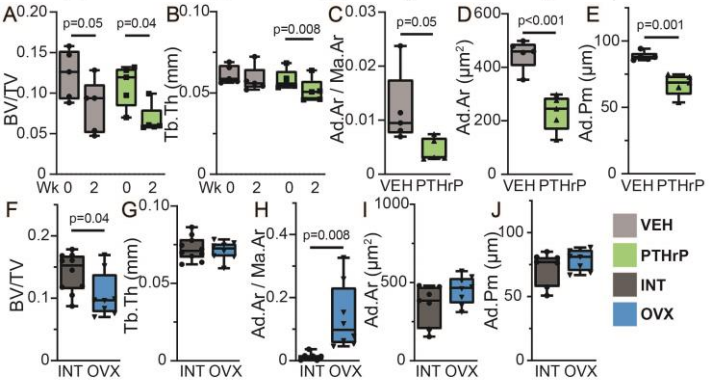

Fig.2 Comparisons of BV/TV, Tb.Th, Ad.Ar/Ma.Ar, Ad.Ar, and Ad.Pm in (A-E) Vehicle and PTHrP infusion and (F-J) Intact and OVX mice

DISCUSSION: Our results demonstrate a non-reciprocal relationship between lactation-induced bone loss and BMAT, with significant reductions in BMAT during lactation and full recovery post-weaning. Elevated serum PTHrP, induced by infusion, led to bone deterioration and significantly lower adipose fraction, size, and perimeter, while estrogen-deficient mice showed an increased BMAd fraction. This suggests that elevated PTHrP levels may drive the unique, concordant changes in bone and BMAT during lactation. We further explored the effects of conditional PPR deletion in BMAds on lactation-induced tissue responses. Despite the evident bone loss, Tb.Th and cortical parameters remained unchanged in the cKO mice, indicating that PPR ablation in BMAds may uniquely influence osteogenic cues during lactation. Surprisingly, BMAT responses did not differ between the WT and the cKO mice. Our previous study indicated that labeled cells constituted about 50% of the total mature adipocytes at euthanasia [1], suggesting the need for an alternative model of Adipoq-CreERT2;PPR^{flox} in future research. Moreover, significantly increased energy metabolism during lactation may impact BMAds independently of PTHrP signaling. Overall, our data confirm a unique, unilateral relationship between bone and BMAT during lactation, with elevated PTHrP as a potential driver. Additionally, PPR ablation in adipocytes may differently impact osteogenic and adipogenic responses during lactation, highlighting a critical area for further investigation.

SIGNIFICANCE: This study elucidates the role of PTHrP/PTH1R signaling in BMAds, demonstrating its critical role on the functional interaction between BMAT-bone, and its impact on maternal bone health.

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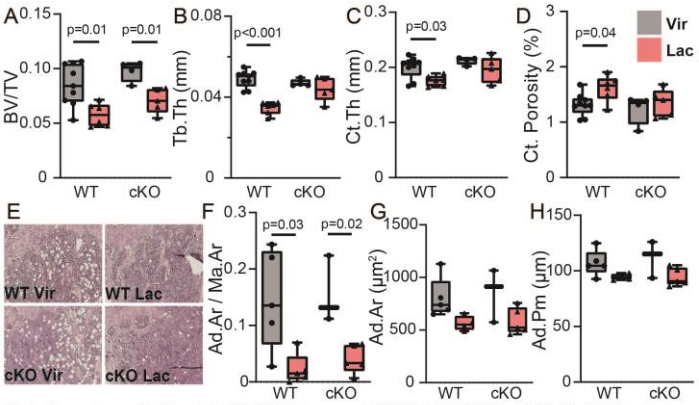

Fig.3 Comparisons of Adipoq-CreER;PPR^{floxed} groups: (A) BV/TV, (B) Tb.Th, (C) Ct.Th, (D) Ct. Porosity, (E) Representative images of BMAT, (F) Ad.Ar/Ma.Ar, (G) Ad.Ar, and (H) Ad.Pm